SUPPRESSOR CELLS IN KIDNEY ALLOGRAFT TOLERANCE

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The state of immune tolerance in organ transplantation is still an enigma more than 20 years since its first experimental induction. It is a rather complex phenomenon implicating several mechanisms such as serum blocking factors, anti-idiotypic antibodies, and the suppressor T cell system.

We had the opportunity in a single kidney transplant institution to study the immunological reactivity of a large and homogenous transplant population against their own specific donors. We observed a decrease of the cellular reactivity of such transplant patients only against their specific donor and in case of transplant tolerance. Cellular reactivity against a third party was normal.

Questioning this loss of reactivity, we were able to show the appearance of a new cellular population in the peripheral blood lymphocytes (PBL) of such patients, post transplant, able to suppress the specific response of the pre-transplant PBL against the specific donor. This is the first study of a large group of patients with some evidence of suppressor cells acting at a helper cell level.

Materials and methods

Patients

Group A tolerant patients: a total of 26 patients in a state of good graft tolerance (serum creatinine ≤ 2mg%) 6 months post-transplantation were studied over the past 2 years.

Group B rejection status: among the 26 patients of group A, 9 underwent a cellular rejection after the period of tolerance and they were also studied at this time (rejection status).

Donor cell freezing and preparation of peripheral blood lymphocytes (PBL)

Donor lymphocytes were prepared from the spleen and frozen. One hundred cc of peripheral blood from each patient was withdrawn on heparin just before trans-
plantation, before the patient had received any immunosuppressive drug. Lymphocytes were isolated on Ficoll Hypaque as described above and frozen. PBL from unrelated control subjects were also prepared and kept frozen.

**Unidirectional mixed lymphocyte culture (MLC)**

The MLC of a recipient PBL to the specific donor spleen lymphocytes was done six months after grafting. Stimulating cells were always obtained from specific donor frozen cells (DF) or unrelated frozen control (CF) and fresh peripheral blood from unrelated controls (C). Stimulator populations were inactivated by administration of 2000 rad ⁶⁰Co irradiation (x) and adjusted to 4 x 10⁶ cells/ml of culture medium (CFx, DFx, Cx). Responding cell populations were patient and control fresh cells (R,C), prepared with the use of Ficoll Hypaque, and thawed cells from patient and controls before grafting (RF,CF) obtained as described above. Responding PBL were resuspended to a concentration of 2 x 10⁶ cells/ml. For mixing experiments, modulator cells were cells from the patient (R) that might alter the response between patient’s cells before grafting (RF) and specific donor or control lymphocytes (DFx or CFx). Modulator cells were resuspended at a concentration of 4 x 10⁶ cells/ml. Cultures were set up in triplicate in microtest plates (Falcon 3040). Each well contained 0.1ml of stimulating cells (4 x 10⁵ cells), plus 0.1ml of the appropriate responding cells (2 x 10⁵ cells), plus 0.05ml of medium. For mixing experiments, each well contained 0.1ml of stimulating cells, 0.1ml of responding cells and 0.05ml of modulator cells (2 x 10⁵ cells). The cultures were incubated at 37°C for 7 days.

**Relative response** the relative response (RR) measures the recipient’s specific response to donor cells (R + DFx) compared to the response to allogeneic control cells (R + CFx) and is calculated: R + DFx/R + CFx or R + DFx/C + DFx. For mixing experiments, the action of recipient’s modulator cells (R) upon reactivity between recipient’s cells before grafting (RF) and specific donor’s cells (DFx) was compared to RF + DFx, RF + CFx or R + CFx. In all experiments the action of modulator cells was also compared to RF + RF + DFx or to RF + RFx + DFx but these results gave the same order of response; thus we will show in our results R + RF + DFx/RF + DFx, and the specificity is expressed by the formula R + RF + DFx/R + RF + CFx.

**Results and discussion**

**Tolerant status (Figure 1)**

PBL from 26 transplant patients (R) were tested against their own specific donor (DFx) or control (CFx) and compared to the reactivity of PBL from control subjects (C). Results are shown in Figure 1. The relative response of specific vs control stimulating cells was 26 ± 4% of the normal response. The relative response of specific vs control responding cells was 23 ± 4% of the normal response. Thus, an allogeneic unresponsiveness could be seen in a specific fashion, i.e. between
Figure 1. Relative response of recipient's lymphocytes (R) stimulated by his own specific donor's lymphocytes (DFx) compared to the response of control's lymphocytes (C) in case of tolerance

the recipient donor pair. This type of reaction was not due to the fact that frozen cells were used as stimulating cells: the RR R + DFx/R + Cx when using Cx where C was fresh PBL from control subjects was found to be 24 ± 4%.

Mixing experiments

Regulatory cells (R) were added to pretransplant PBL of patients (RF) stimulated by the specific donor (DFx), or frozen cells from control subjects (CFx). Addition of R lowered the response to 38 ± 8% when R + RF + DFx is compared to RF + DFx, and respectively to 43 ± 8% and 40 ± 5% when compared to RF + CFx and R + RF + CFx. Thus the amount of suppression is about 60%, and also specific for the recipient donor pairs. We compared R + RF + DFx to RF + RF + DF and to RF + RFx + DFx in order to compare a similar number of cells in each well. Results were similar to the above, but with a slight increase of suppression (70 ± 6% when using RF + RF + DFx) due to doubling the number of responding cells. A cell crowding effect could be ruled out. We have to note that two patients had no demonstrable suppressor cells.
Rejection status (Figure 2)

Eleven cases of transplant patients with rejection episodes, more than 6 months post grafting were tested in the same way. The RR R + DFx/R + CFx and R + DFx/C + DFx gave respectively 277 ± 69% and 170 ± 70% of a normal response. Thus, in this peculiar case of rejection, no unresponsiveness could be seen; but rather a mitogenic effect against the specific donor. A wide variation between each individual RR explains the large S.E.

As a matter of fact, the literature dealing with the cell-mediated reactivity of TP against their own specific donor is highly controversial. MLC have been found to be either low [1,2] or normal [3,4]. On the other hand, the specific allogeneic response, monitoring the status of transplant patients (TP) was reported to be poor in cases of cellular rejection [4] in contrast to cell-mediated lympholysis (CML) which gave a higher rate of correlation [4].

Suppressor cells in human transplantation tolerance have not been extensively demonstrated. Three groups have shown that the in vitro development of cytotoxic T cells as a result of proliferation in MLR is impaired in long term survivors [5–7]. This suppression was shown to be specific for antigens of the donor when recipient cells were added to other MLR-CML mixtures. But these reports showed an

Figure 2. Relative response of recipient's lymphocytes (R) stimulated by how own specific donor's lymphocytes (DFx) compared to the response of control's lymphocytes (C) in case of rejection
impairment of CTL generation (equivalent of T cells bearing the LY 2+3+ phenotype in the mouse) by suppressor cells, not affecting the generation of helper T cells (equivalent of the LY 1+ phenotype in the mouse). It seems that we are the first to show in a large group of patients that suppressor cells could act also at a helper cell level in a system where pre and post transplant lymphocyte reactivity against the specific donor could be compared. On the other hand, in mixing experiments we use only two MHC different populations (R and D), avoiding an allogeneic effect resulting from 3MHC different populations mixed in the same well. We have to add that in our system, the generation of killer cells ‘in vitro’ against the specific donor was also impaired although a normal response vs a third party was seen.

References